Leveraging Chemically Modified Single-stranded RNA Editing Oligonucleotides For Future Therapeutic Applications

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Introduction

Adenosine deaminase acting on RNA (ADAR), a key RNA editing machinery

Among emerging genetic medicines, ADAR-based RNA editing is poised to become a major therapeutic platform. ADAR, a naturally occurring enzyme in all human cells, edits adenosine (A) to inosine (I) within double-stranded RNA (**Fig. 1**, left panel). This A-to-I editing is a common biological process essential for neuronal maturation, immune self-recognition, genome stability, and RNA processing.[1,2]

Axiomer™ Editing oligonucleotides (EONs)

ProQR's Axiomer platform harnesses the body's natural ADAR RNA-editing process using chemically modified Editing Oligonucleotides (EONs). EONs specifically bind to a target single-stranded RNA region to form a double-stranded structure that recruits endogenous ADAR enzymes (**Fig. 1**, right panel). ADAR then converts specifically one adenosine (A) to inosine (I), which is read as guanosine (G) during translation. This precise editing can correct disease-causing RNA mutations or modulate protein function to prevent or treat disease.

Natural ADAR editing *(A-to-I)* | **Editing Oligonucleotide (EON) directed therapeutic editing** *(A-to-I)*

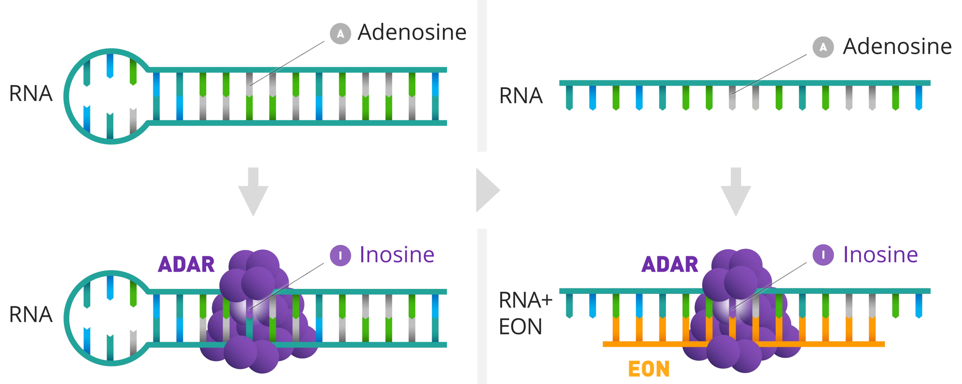

Figure 1. *On the left,* ADAR enzymes naturally act on double stranded RNAs and perform a specific A-to-I editing, changing Adenosine (A) to Inosine (I). *On the right,* by learning from this natural process, ProQR has developed editing oligonucleotides (EONs).

Objectives

To understand the breadth of Axiomer applicability and therapeutic potential by assessing EONs designed to correct G-to-A mutations and multiple forms of protein modulation.

Results

Modulate protein function

AX-0810 *RNA editing therapy targeting SLC10A1 (NTCP) for Cholestatic Disease*

Cholestatic diseases are a group of diseases with wide spectrum. Despite their diversity, cholestatic diseases all share a common biological pattern: the **toxic accumulation of bile acids in the liver**.[3,4] AX-0810 is a therapeutic approach targeting NTCP, the main transporter involved in bile acid re-uptake from the bloodstream in the liver. (**Fig. 2**) Modulation of NTCP could lead to **hepatoprotective effects and become a disease modifying therapy**.

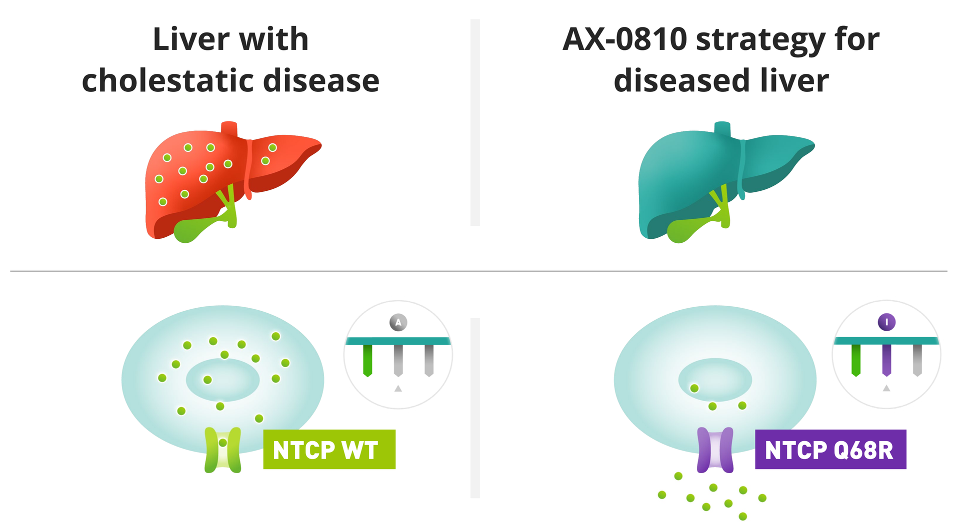

Figure 2. AX-0810 approach modulating NTCP channel to address toxic overload in Cholestatic Diseases

Following treatment of mice *in vivo*, Axiomer resulted in approximately up to 15% editing reaching expected NTCP modulation with more than 2-fold changes vs. baseline in a biomarker of interest. (**Fig. 3**)

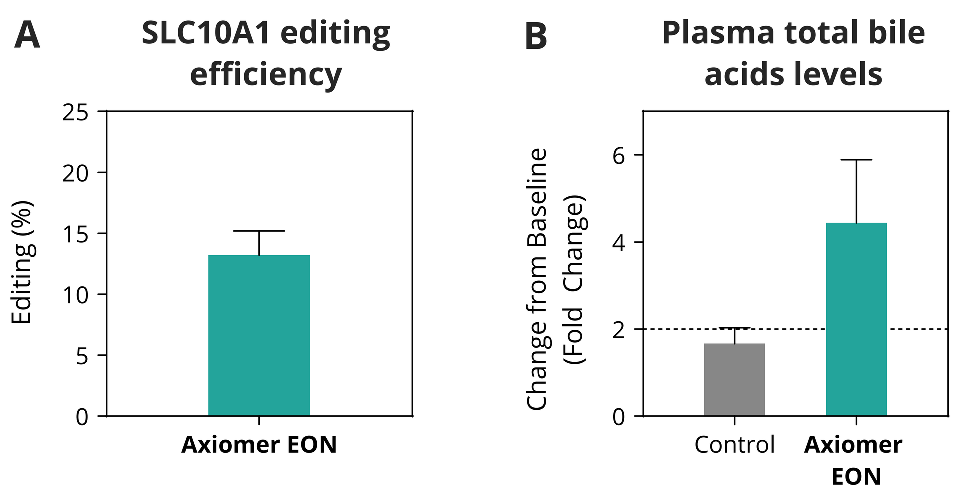

Figure 3. *SLC10A1 RNA* editing (**A**) and impact on plasma total bile acids levels (**B**) following Axiomer EON treatment in mice *in vivo*. Treatment conditions: N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, editing assessed with ddPCR. Data are represented as mean, SEM.

Correct mutations

AX-2402 *RNA editing therapy targeting MECP2 for Rett Syndrome*

MECP2 gene, encoding methyl-CpG binding protein 2 (MeCP2), is a master epigenetic regulator of gene expression essential for neuronal maturation and function. Mutations leading to loss of MeCP2 function cause Rett syndrome, a **devastating and progressive neurodevelopmental disorder**.[5] AX-2402 approach can **restore physiological levels of functional MECP2** by converting R270X to R270W, a variant indistinguishable from wild-type (WT). (**Fig. 4**)

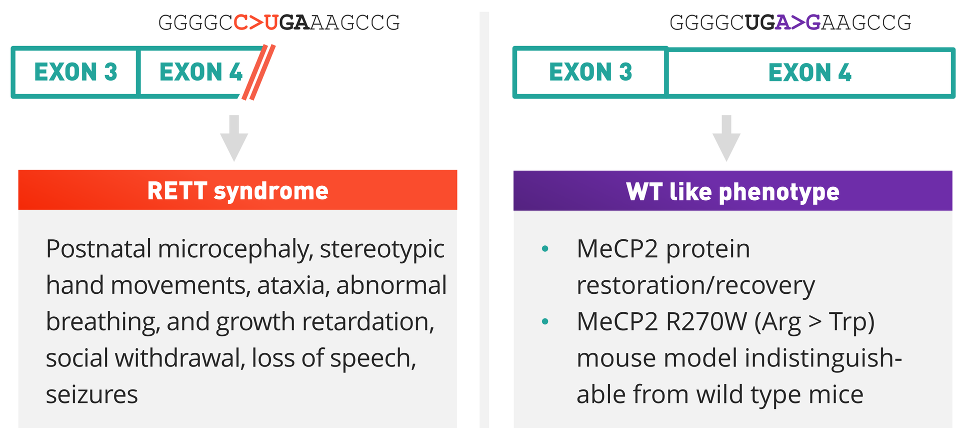

Figure 4. AX-2402 approach converting R270X to R270W to address loss of MeCP2 function in Rett syndrome

Up to 80% RNA editing of the R270X MECP2 was achieved in patient fibroblasts leading to an increase in MeCP2 protein activity up to ~60% of WT. (**Fig. 5**)

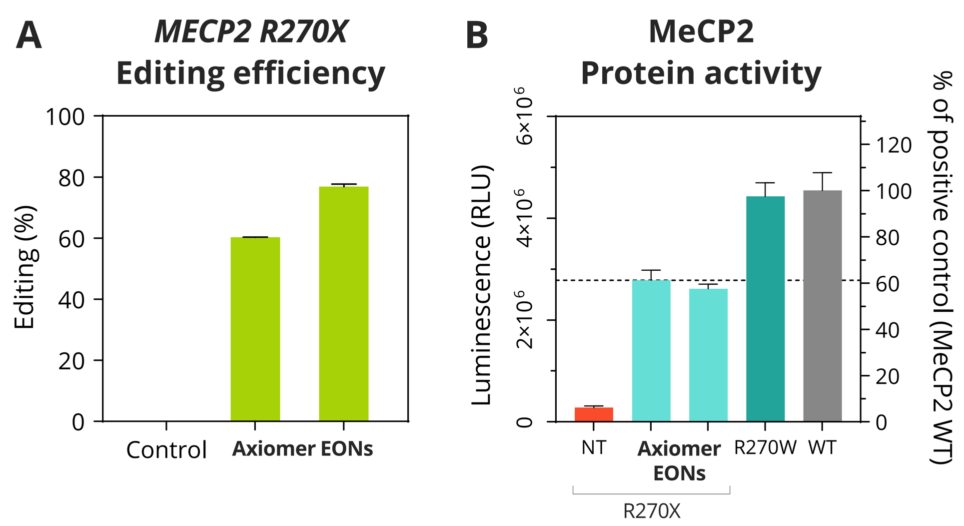

Figure 5. (**A**) RNA editing of R270X *MECP2. Treatment conditions:* Patient fibroblasts, transfection at 25 nM (N=2, 48 h) (**B**) MeCP2 protein activity. Treatment conditions: *MeCP2 Reporter Activity Assay,* cells transfected with plasmids encoding MECP2 R270X, MECP2 R270W, or MECP2 WT (reverse transfection, 24 h, 100 ng/ml, Turbofect), followed by EON forward transfection (100 nM, 48 h, RNAiMax; N=4). Data are represented as mean, SEM.

AX-2911 *RNA editing targeting PNPLA3 for Metabolic Dysfunction-Associated Steatohepatitis (MASH)*

PNPLA3 (patatin-like phospholipase domain-containing 3) I148M is a variant **commonly reported in the MASH population worldwide** (20-60% of the patients) and is known as associated risk factor.[6,7] Approximately 8 million individuals in US and EU are homozygous for the 148M variant. Axiomer EONs have the potential to change the Methionine into a Valine **bringing the PNPLA3 protein back to a WT-like functional conformation**. (**Fig. 6**)

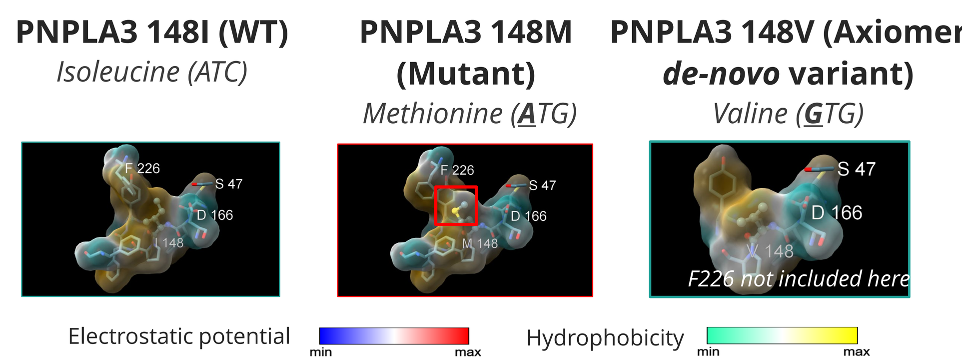

Figure 6. AX-2911 approach converting PNPLA3 protein back to a WT-like functional conformation

Editing of PNPLA3 in PHH reached approximately 50% and Axiomer EONs led to a decrease in intracellular lipid droplets. (**Fig. 7**)

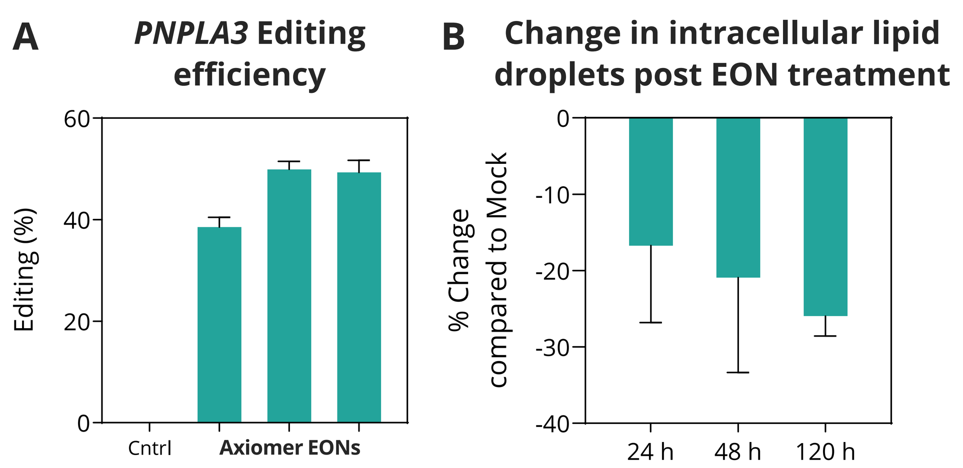

Figure 7. (**A**) RNA editing of PNPLA3 in PHH. *Treatment conditions:* 100nM EON, transfection, 72h, dPCR, n=3 (**B**) Change in intracellular lipid droplets post PNPLA3 148V EON treatment in HepG2. *Treatment conditions:* Bodipy/DAPI stainings, 5µM EON, transfection, exposure to linoleic acid, n=2. Data are represented as mean, SEM.

Change protein interactions

AX-1412 *RNA editing therapy targeting B4GALT1 for cardiovascular diseases (CVDs)*

B4GALT1 p.Asn352Ser variant was identified in human genetics as a protective factor, known to significantly reduce the risk of coronary artery diseases.[8] The AX-1412 program is designed to introduce this protective variant at the RNA level, aiming to facilitate clearance of lipoproteins from the bloodstream via a reduction in galactosylation (**Fig. 8**)

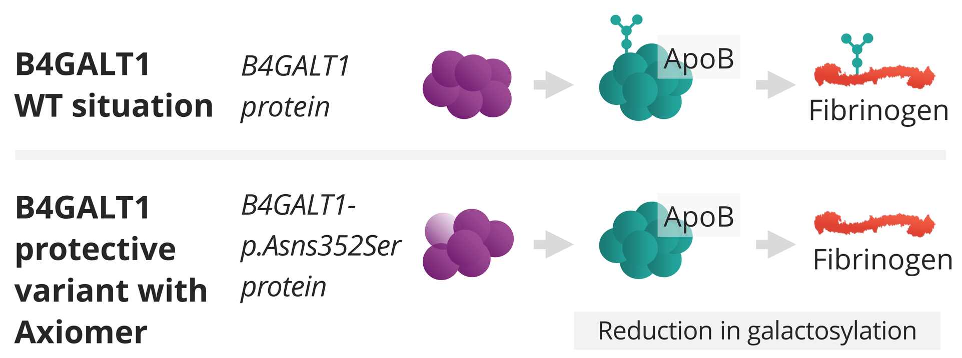

Figure 8. AX-1412 approach introducing a protective variant reducing galactosylation of lipoprotein

Treatment with Axiomer EON led to a reduction in ApoB, LDL-c and fibrinogen (amongst other biomarkers) in E3L.CETP mice model (**Fig. 9**)

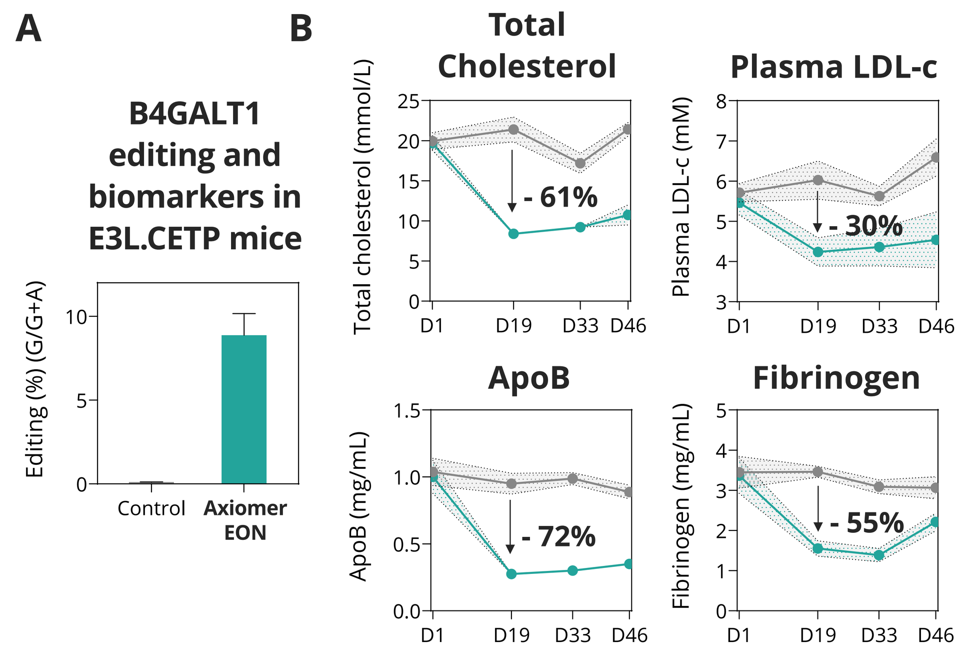

Figure 9. (**A**) RNA editing of B4GALT1 in E3L.CETP mice., *ddPCR* (**B**) Change in total cholesterol, LDL-c, ApoB and fibrinogen. *Treatment conditions: N=10, 2mg/kg, LNP formulation, IV Q1W, D46.* Data are represented as mean, SEM.

Conclusions

- **Axiomer EONs are designed to recruit and direct endogenously expressed ADARs.**
- **Axiomer EONs have the potential to correct RNA with disease-causing mutations back to functional.**
- **Form, modulate protein expression, or alter a protein so that it will have a new function that helps prevent or treat disease.**
- **Across various targets, Axiomer EONs have demonstrated editing activity and impact on biomarkers of interest, highlighting their potential for future therapeutic development.**

Literature : 1. Dewey FE et al. N Engl J Med. 2017 Jul 20;377(3):211-221; 2. Hajji K, et al. RNA. 2022;28(10):1281-1297; 3. Zeng J, Fan J, Zhou H. Cell Biosci. 2023 Apr 29;13(1):77; 4. Trauner M, Fuchs CD. Gut 2022;71:194–209; 5. Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7; 6. Romeo S, et al. Nat Genet. 2008 Dec;40(12):1461-5; 7. Salari N, et al. BMC Endocr Disord. 2021 Jun 19;21(1):125; 8. Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227.